HUMMEL

Cytology of the Endosperm of Ricinus

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CYTOLOGY OF THE ENDOSPERM OF RICINUS

BY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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Introduction.

Our knowledge of the cytology of the endosperm of Ricinus and other seeds is based largely on the early investigations of Koeppen, (8) Peters, (9) and Raciborski (11). The subject was taken up anew with the hope of acquiring a fuller knowledge of the dormant cell and the cellular changes that accompany the process of germination, by the use of modern methods and the application of our more comprehensive view of cellular phenomena.

I have made a careful cytological study of many seeds of Ricinus in the dormant condition and in various stages of germination. My attention has been directed more particularly to the cells of the endosperm; but for purposes of comparison, I have also studied the cells of the embryo. I have made no attempt to ascertain the cytological changes that accompany the formation of the seed.

Material and Method.

The seeds that were used in these studies were, for the most part, kept in the laboratory in a dormant condition for about a year. Some fresh ones were used also, but they differed in no wise from those above noted. In preparing the seeds for germination they were imbedded in moist sterile sawdust. From some of the seeds the testas were removed before imbedding. The vessel containing the imbedded seeds was kept in the dark in the greenhouse; no attempt being made to keep the temperature and moisture constant. In some instances the endosperm was split into halves parallel to the cotyledons. After carefully removing the cotyledon from one half, both halves were placed under favorable conditions for germination.

Again small pieces of endosperm, some with, others without, embryo attached, were placed under favorable conditions for germination. In all cases accurate records of the time of germination have been kept, but no significance can be attached to them, owing to the great variation in the individual seeds. I have attempted to trace the sequence of changes that accompany germination, and in order to do so I have been compelled to disregard, almost entirely, the element of time.

In order to properly fix the protaplasmic constituents of the cell, together with their inclusions, and to provide a means of contrasting the effects of different fixing agents, it has been found necessary to use the following killing and fixing agents.

Absolute Alcohol ------------ 6 Parts
Glacial Acetic Acid ---------- 1 Part
Chloroform ------------------ 3 Parts

Absolute Alcohol ------------ 2 Parts
Glacial Acetic Acid ---------- 1 Part

Flemming's Fluid.

Osmic Acid ------------------ 1 Gram
Chromic Acid ---------------- 3.6 Grams
Glacial Acetic Acid -------- 24 Cubic Centimeters
Distilled Water ----------- 432 Cubic Centimeters

Corrosive Sublimate --------- 5 Grams
Picric Acid Crystals -------- 1.3 Grams
Glacial Acetic Acid --------- 5 Cubic Centimeters
Alcohol 50% --------------- 100 Cubic Centimeters

Alcohol -------------------- 95%

The seeds intended for study were cut (transversely or longitudinally) into slices about two millimeters in thickness and dropped immediately into the killing and fixing agent. The material for sectioning was prepared for paraffin (52 C.) infiltration in the usual way, chloroform being used as a clearing agent. The sections cut varied from five to twenty microns, the usual thickness being 5 microns.

Successful staining of the cell constituents and their inclusions cannot be obtained by the use of a single stain. It has been found advantageous to use the following stains:

Delafield's Haematoxylin
Haidenhain's Iron Alum-Haematoxylin
Flemming's Triple Stain of Safranin, Gentian-Violet, and Orange G.
Michaelis' Scharlach R. (A saturated solution in 70% alcohol.)
Alcannin
Methelyn Green

In the preparation of the seed for sectioning, by either the paraffin or celloidin process, the oil was unavoidably removed from the cell. This made it impossible to study the oil in its normal relations. I therefore found it necessary to make free hand sections from fresh material. These sections were immediately stained for several hours in the saturated solution of Scharlach R. and then mounted in glycerin and glycerin jelly.

The study of the microtome sections was in all cases supplemented by a study of free hand sections from living material.

Gross Structure.

In a Ricinus seed that has remained dormant in a comparatively dry atmosphere for some time, the endosperm has shrunken away from the wall of the testa and has formed a cavity about the embryo. (Fig. 1).

When germination begins the endosperm grows (1) so that it soon completely fills the space within the testa and presses the two cotyledons close together (Fig.2). Soon the testa is broken open, and the process of growth continues until the endosperm often times more than doubles its original dimensions (Fig.3). The embryo, too, grows during germination and soon after the testa is broken, the radicle pushes its way through the endosperm and continues to elongate. After a few days it reaches a length of several inches and secondary roots begin to form. When these have attained a length of approximately two inches, the endosperm reaches its maximum size (Fig.3). The cotyledons continue to grow during germination, and when the endosperm ceases to enlarge, they pierce its margins (Fig.4). A little later, when the hypacotyl elongates, the cotyledons are pulled out of the endosperm and are soon raised above the soil. From the time the endosperm ceases to enlarge until the cotyledons are removed from it, there is a gradual change taking place. The endosperm becomes thinner, more transparent and elastic. It sometimes remains in contact with the cotyledons until there is nothing left of it but a very thin skeleton, slimy in nature and teaming with bacteria. When the cotyledons emerge from the endosperm they generally show the presence of a red color on both surfaces. This red color has been found in the cotyledons before they are withdrawn from the endosperm, but always in stages where

the endosperm has ceased to grow. No color has ever been noted in the cotyledons in the earlier stages of germination.

In 1877 Van Tieghem (14) found that parts of the endosperm of Ricinus would grow without the embryo being present. He found that such pieces of endosperm, when subjected to favorable conditions for germination, would double in length in a month. In 1890 Green (3), working along similar lines, found that the endosperm grew more rapidly when the embryo was in contact with it. This he interpreted as being due to a physiological condition caused by the presence of the embryo acting as a stimulus to the protoplasm of the endosperm cells. At this time, as shown by the quotation that follows, he did not believe that the endosperm was affected by excretion from the embryo. "It is difficult to suggest an adequate explanation of this action. As already shown, it is not caused by the formation and subsequent excretion of the glyceride ferment. Nor does it appear that the embryo excretes anything that may bring about the later changes which the ferment does not effect." In a later article Green and Jackson, (4) referring to the changes caused by the presence of the embryo in germination, make the following statement: "No satisfactory explanation of these phenomena was forthcoming at the time that they were observed, but the discovery that the tryptic enzyme is secreted by the cotyledons affords one."

I repeated in slightly modified form, the experiments of Van Tieghem and of Green, in order to observe the germination of the mutilated endosperm. A large number of endosperms were divided into halves parallel to the cotyledons. One half of each endosperm was allowed to germinate with the cotyledon in contact with it; the other half was placed beside it, for germination, after the cotyledon was removed. Nearly two weeks elapsed before any differences

were observed in the development of the two halves of the endosperm. They both grew at the same rate nearly doubling their dimensions. At about the end of two weeks, the halves of the endosperm from which the cotyledons had been removed, began to show signs of decay. This decay increased and in a short time these halves were dead and disintegrating. The halves of the endosperms in which the cotyledons remained in contact continued in a healthy condition for some time after the others began to decay. However, no further increase in growth could be detected. Later I germinated some small pieces of endosperm cut from various parts of the seed, some with pieces of embryo attached, others without. In all cases, even when the pieces were very small, there was a uniform manifestation of life and growth both in the embryo and in the endosperm.

It is evident that my results do not correspond with those which Green obtained, with endosperm that had the cotyledons removed. I have been unable to get any results which indicate that the presence of the embryo has any influence upon the growth of the endosperm during germination. On the other hand, I must state that while the embryo does not affect the growth of the endosperm, it does in some way prolong the life of the endosperm. This is probably accomplished by the excretion of an acid by the embryo which has a germicidal action.

Cellular Differentiation.

The cells of the endosperm of Ricinus are large, slightly irregular in outline, and nearly uniform throughout the endosperm. In the dormant condition there are generally several layers of cells lying next to the embryo, which have lost their cell contents and

consist simply of crushed layers of cell walls. Toward the periphery of the endosperm there is a gradual change in the cell contents, so that the outermost layer of cells differs from the central cells in having much smaller aleurone grains, more cytoplasm, a nucleolus, and a large nucleus (Fig.5).

During germination the cells grow to nearly twice their original size, and in so doing, they form an increased amount of intercellular space (Figs.8 to 11). In some places, especially close to the embryo, the cells are pressed together by their growth so that they form rows radiating from the embryo. The changes that accompany germination are remarkably uniform throughout the endosperm. One would naturally expect the endosperm to show signs of life first in some particular region and then this renewed activity spread to other parts. Such is not the case, for the cells throughout the endosperm change simultaneously,with the exception of a few scattered cells which may be in advance or behind the others in their development. This is arather significant fact, for it indicates that the endosperm is not dependent upon the embryo for its stimulus for growth. There is no cell division taking place in the endosperm during germination. The growth that is manifest is simply due to the enlargement of the cells present.

The dormant cell consists of a thin cellulose cell wall, within which is found a more or less indistinct nucleus and mass of cytoplasm. In the meshes of the cytoplasm are found many aleurone grains and a great quantity of oil.

The Cell Wall.

Green (3) in describing a dormant cell of the endosperm of

Ricinus says: "There is no great amount of cellulose, the cell walls being thin and the cells of a fair size." My observations are in accord with his statement. The cell walls are very thin in the dormant cells, and remain so throughout germination. The cells do not appear turgid in thedormant condition, but show signs of partial collapse. In the later stages of germination they are much larger, as has been stated before; this indicates that the cellulose walls have grown, by having new material added to them.

Green (3) also makes the following statement concerning cell walls: "On examining some of the endosperms after a prolonged period of germination, carried indeed so far that there was only a thin, almost slimy casing over the cotyledons, the cells were found to be empty of solid contents, except a thin layer of protoplasm, and the cell walls were disintegrating and disappearing." In only a very few instances did I find cell walls disappearing in the late stages of germination. On the other hand, I found, generally, that the cell walls were still in tact after practically all the cell contents had disappeared and the endosperms were shriveling. I am inclined to think that in the few instances noted above, the disintegration of the cell walls was due to bacterial action and not to an enzyme secreted by the seed.

The Oil.

In 1885, Harz (7) made a quantitative test of the oil contents of many dormant Ricinus seeds and found that the amount varied from 40% to 68%. In 1890, Green (3) records an average of 58% for the seeds examined. It is evident from these records, that there is a large amount of oil present in the dormant seeds of Ricinus. Many

investigators have attempted to discover the processes by which this oil is utilized by the germinating seed. The last one to work upon this subject was Green (3 & 4). I have not attempted to verify his results along this line, nor to add anything new to the admirable work already done.

I have found that there is, in the dormant endosperm cell, a mass of oil surrounding the aleurone grains and lying in the outer region just within the cell wall. In fact all parts of the cell, not occupied by other cell constituents and inclusions, seem to be filled with the oil. Whether this is stored in the meshes of the cytoplasm or in specially formed pockets, I am unable to state. During germination, as Sachs observed in 1859, (12) the oil gradually disappears. At the time the cotyledons are withdrawn from the endosperm, there are only slight traces of oil remaining and these are in the meshes of the cytoplasm near the nucleus.

The Aleurone Grains.

In 1855 Th. Hartig, (6) in describing the proteid bodies, which he found in several kinds of seeds, called them aleurone grains. Strasburger (13) describes the aleurone grains of Ricinus as protein grains enclosing albumen crystals and globoids. In 1880, Vines (15) makes the following statement in regard to aleurone grains: "Since their first discovery the aleurone-grains have been usually regarded as aggregations of reserve-proteids laid up in the seed for the nourishment of the embryo, and it is doubtless their fate to be more or less absorbed."

In 1887, Green (2) makes the following statement about the changes that accompany the germination of the Lupin seed: "The histolog-

ical changes accompanying these chemical ones are interesting. In the resting seed the proteid matter is present in the form of aleurone grains, which are small, round bodies imbedded in a network of protoplasm. Their outlines are sharp, and they occupy about half the whole space of the cell. In a seed in which germination has just commenced the grains are found to have become larger, probably from taking up water and the protoplasmic network is consequently more compressed. A later stage shows the grains to have a much less distinct outline, though they retain their almost spherical shape. They are now studded with sparse granules, and appear to be dissolving from within outwards. When the radicle has attained a length of about 1.5 inches the disintegration of the grains is very marked, and the protoplasm contains empty spaces in which they formerly lay. In the actively growing seed, the radicle being 2 or 3 inches long, there is little left except the meshwork of protoplasm which, now relieved from tension, is seen to be very loose, and to contain large vacuoles."

Our knowledge of the origin of the alemone grains in Ricinus, is largely based upon the early investigations of Pfeffer (10).

When free hand sections of the dormant seeds of Ricinus were mounted in glycerine, without previous killing, they showed some aleurone grains to be as figured by Strasburger (13). They are large and numerous, nearly spherical in shape, and each one contains, along its outer margin, one or more spherical globaids, and near its center one or more crystalloids. The crystalloids vary in size as well as in outline. They often appear as a hardened central part of the aleurone grain without any distinct border; others have a distinct outline and appear as separate cubical bodies; again others appear as a number of small beads in the center of the al-

eurone grain.

When I studied sections that had been killed and fixed, I found that different killing fluids affected the aleurone grains quite differently. When Flemming's Fluid was used for killing, the aleurone grains showed no differentiation into glaboids and crystalloids but were uniform throughout, and slightly granular in appearance (Fig. 8). Sections of endosperm that had been killed with Corrosive Sublimate, Picric Acid, Acetic Acid, and Alcohol, showed the globoids distinctly, but the crystalloids were not visible in the body of the aleurone grain which appeared homogeneous (Fig.6). In sections which had been killed with Absolute Alcohol, Chloroform, and Acetic Acid, the body of the aleurone grain had apparently disappeared and there was but little of it remaining except the globoid and many small,bead-like crystalloids (Fig.7). I have made no attempt to trace the fate of the globoids and crystalloids through the stages of germination, but have followed very carefully the changes that have taken place in the aleurone grain as a whole (Figs. 8 to 11).

Some of the aleurone grains of dormant cells contain small regions where they are slightly more transparent than in the other parts. Generally in the center of one of these transparent regions I could see a very small opaque granule almost invisible (Fig.8). This transparent region may mark the beginning of enzyme action, at least I am unable to account for it in any other way.

When germination begins, the aleurone grains slowly increase in size. While thus enlarging, solution is taking place from within outwards and this continues until the whole grain finally disappears (Figs.8 to 11). This process is very similar to the one Green has described in the germinating Lupin cotyledons. Sometimes

in the growth of the aleurone grains they coalesce to form a large, irregular mass. The last traces of the aleurone grains are found in the outer part of a large vacuole in the cytoplasm. It is not surprising to find a vacuole arising from an aleurone grain, for Pfeffer (10) has found that in the process of forming the aleurone grains are laid down in the vacuoles of the cytoplasm. The last of the aleurone grain disappears at about the time the cell reaches its maximum size.

The breaking down of the aleurone grain is evidently due to enzyme action which is closely associated with the life processes of the cytoplasm in which the grain is imbedded. I have made no attempt to ascertain the nature and origin of the enzymes that may be present. They may arise from within the aleurone grain or they may be a secretion from the investing cytoplasm.

The Cytoplasm.

In 1865, Gris, (5) after working with Ricinus and several other seeds, came to the conclusion that during germination the endosperm was the seat of an independent life. In 1905 Green (4) states that Mr. Biffin, in the Cambridge Botanical Laboratory, has notedfor the first time the fact that on germination the amount of protoplasm increases very markedly in the endosperm cells of Ricinus.

As has been stated before, there is much more cytoplasm in the peripheral cells of the endosperm during the dormant period than there is in the central cells (Figs.5 and 8). Why this is true, I am unable to state definitely, but am inclined to think that the atmospheric relations are responsible for it. The central cells in their dormant condition have very little cytoplasm in evidence.

During germination the amount of cytoplasm gradually increases until it reaches a maximum at about the time the last of the aleurone grains disappear. It then begins to decrease in amount and at the time the cotyledons are removed from the endosperm it is present only as a very thin lining to the cell wall (Figs.8 to 15). From the time germination begins until the cytoplasm has practically all disappeared there are evidences of continuous metabolism within the cell. I am inclined to think that my results indicate that Gris was not far from right in his conclusion as previously stated. In general my results also coincide with those of Mr. Biffin, as stated by Green, in regard to the increase of protoplasm in the germinating endosperm.

The Nucleus.

In 1891 Peters (9) found that the nucleus as well as the nucleolus increased in size during the germinating period of Ricinus. He explained this fact as probably due to the increased supply of food furnished by the breaking down of the aleurone grains.

With the nucleus as with the cytoplasm there is a marked difference in the peripheral cells of the endosperm as compared with those in the interior. The nuclei in the peripheral cells, in the dormant state, are at about the same stage of development as are the nuclei of the central cells after they have been germinating for about twenty-four hours (Figs. 5 and 10).

In the central dormant cell the nucleus is very small and indistinct Figs. 6 to 8). Only after examining the cells late in germination and following the nucleus back to the dormant cell, could I be sure that I had identified the nucleus properly. As germination proceeds the nucleus grows. At first it is homogeneous, but

after the appearance of one or more nucleoli it becomes more differentiated. Soon the reticulum becomes apparent and chromatin granules appear irregularly distributed through it. The nucleus reaches its maximum size at about the same time the cytoplasm reaches its maximum. It then becomes vacuolated and gradually grows smaller until it finally disappears (Figs. 8 to 15).

The nucleolus first appears as a very small body. It grows as do the other parts of the protoplasm and reaches its maximum size at about the same time the nucleus does. It then becomes vacuolated and lies within a "Hof;" soon it gradually grows smaller and often contains more than one vacuole. Finally it disappears entirely just before the nucleus disappears (Figs. 10 to 15).

The Embryo.

In all sections of endosperm that I studied I had present parts of the embryo. All that has been said concerning the endosperm cells of the central region of the endosperm both in their dormant condition and while growing may be applied to the embryo cells as well. They are much smaller than the cells of the endosperm, but contain the same kinds of stored food materials and grow in the same way (Figs. 16 to 19). Instead of losing their protoplasm after they cease growing, as the endosperm cells do, the embryo cells retain their protoplasm and divide to give rise to new cells. As cell division continues in the cotyledon of the embryo, the characteristic tissues of a leaf soon appear. A little later, there appears in the epidermis of the cotyledons large, oval cells (Fig. 20). In free hand sections, unkilled, these oval cells, when they first arise, appear to be filled with a colorless substance homogeneous

or granular in nature. A little later there appears a red pigment within these cells which soon completely colors the cell contents red. As long as the cotyledons are in complete darkness no red color appears in these oval cells. I have chosen to speak of these cells as chromogen cells because they contain the red color which I have described before. They arise about the time the aleurone grains have completely disappeared and the cytoplasm of the endosperm cells is beginning to decrease. They appear in equal numbers on both surfaces of the cotyledons. When stained with the triple stain they appear as red cells and are easily distinguished.

In 1905, Green and Jackson made the following statement (4) in discussing the "Nutrition of the Embryo" of Ricinus. "In the course of the researches made by Mr. Biffin which have already been referred to, he found that the epidermis of the young cotyledons contained cells, occurring at short intervals, which stained quite differently from the rest, and were full of granular contents. We prepared a large number of cotyledons from seeds in course of germination, taking them at an early stage when it was just possible to separate them clearly from the endosperm. They were then washed carefully in warm, distilled water till all organic matter was removed from their surfaces. Each cotyledon was then cut in half along the mid-rib. One set of halves was dipped for a moment in boiling water. The two sets were put into a solution of the globulin of the seeds prepared by dissolving it from the seed in 10 per cent solution of common salt and precipitating it by strong alcohol. The tubes containing them were put for a few hours into an incubator at 30 C. At the end of this time the uninjured epidermis had produced such a change in the globulin that the solution gave a vivid reaction for tryptophane on addition of a little chlorine water. The contents of the other tube were unchanged. The presence

of trypsin in the cotyledonary epidermis was consequently proved. An extract of the cotyledons gave the same result. Taking these experiments in conjunction with Mr. Biffin's observations, there can be little or no doubt that the special cells alluded to secrete the trypsin."

There can be no doubt that the special cells alluded to in the above quotation were the chromagen cells which I have described. If this be true, then Green and Jackson (4) were experimenting with cotyledons which had not yet developed the cells observed by Mr. Biffin, for I have found that these do not arise until much later in germination. It is evident that Green and Jackson (4) have been entirely mistaken in attributing to these cells, observed by Mr. Biffin and myself, the power of excreting trypsin.

Summary.

What has been said concerning the cytology of Ricinus may be summarized as follows:

The growth in the endosperm in the process of germination, is due to the nearly uniform enlargement of the individual cells and to the increased amount of intercellular space. There is no cell division during the process of germination.

The central dormant cells of the endosperm contain a large amount of oil, many aleurone grains with globoids and crystalloids, a small amount of cytoplasm, and a very small, indistinct nucleus.

During germination an endosperm cell changes as follows. The cellulose wall grows and persists until broken down by bacteria. The oil gradually disappears. The aleurone grains grow as they dissolve from within outward. Soon they completely disappear and their place is occupied by a vacuole. The cytoplasm increases in

amount until the disappearance of the aleurone grains, then it gradually becomes less until only a thin lining is left just within the cell wall. The nucleus grows, forms a reticulum and chromatin matter, and reaches its maximum size. It then becomes somewhat vacuolated and decreases in size until it finally completely disappears. The nucleolus is not present at first, but soon appears and grows to a maximum size. It then forms a "Hof" and becomes vacuolated. Soon it gradually becomes smaller and disappears just before the nucleus. The cell, the cytoplasm, the nucleus, and the nucleolus all reach their greatest development at the time the last of the aleurone grains are disappearing.

Until the aleurone grains have disappeared the changes in the embryo and in the endosperm are practically the same. The embryo cells then continue to remain active and divide to form new cells and new tissue.

A little after the disappearance of the last of the aleurone grains in the endosperm, chromogen cells arise in the epidermis of both surfaces of the cotyledons. Green and Jackson have wrongly taken these, from Mr. Biffin's description, to be trypsin secreting cells.

General Conclusion.

It seems evident, from what has been said, that the metabolic processes in the endosperm cells of Ricinus during germination are carried on wholly independent of the embryo. The embryo, however, absorbs some of the products from the endosperm cells and also acts in the capacity of a germicidal agent. The oil and the aleurone grains are broken down by the living processes of the endosperm cells aided by enzyme action. The products so derived are used by

the living parts of the cell to cause its renewed activity and growth. Until the embryo cells have consumed all of their own reserve material, as aleurone grains and oil, they do not draw upon the food in the endosperm. Since it is true that the aleurone grains and the oil disappear from the endosperm and embryo at about the same time it seems evident that very little of the material of the endosperm is used by the embryo before it has first become a part of the trophoplasm of the endosperm cell. After each cell has consumed all of its available reserve material, both endosperm and embryo cells-- the embryo cells seem to thrive at the expense of the endosperm cells. The material taken from the endosperm by the embryo cells is simply from the living parts of the cells, and not, as has generally been supposed, from the broken down reserve food directly. Since this food, so taken up by the embryo cells,has to pass through many cell walls it is evidently in solution.

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Explanation of Figures.

Figures from 1 to 4 inclusive are free hand drawings magnified two times. All other figures were drawn with a camera lucida, a 1/12 oil immersion lens, and a one-inch occular were used. (Bausch and Lomb instruments were used.)

Abbreviations.

A.	Aleurone Grains	Ep	Epidermis
Ca.	Caruncle	G.	Globoid
Ch.	Chromatin	I	Intercellular Space
Chro.	Chromogen Cell	N.	Nucellus
Cr.	Crystalloids	Nu.	Nucleus
C.W.	Cell Wall	Ns.	Nucleolus
Cy.	Cytoplasm	O.	Oil
Em.	Embryo	T.	Testa
En.	Endosperm	V.	Vacuole

When not otherwise stated the cells figured below were drawn from sections killed with Flemming's Killing Fluid and stained with Flemming's Triple Stain.

Figure 1. A section through a dormant seed.

" 2. A section through a germinating seed just before the testa opened.

" 3. A section through a germinating seed that has reached its maximum size.

" 4. A section through a germinating seed just before the cotyledons are withdrawn from the endosperm.

" 5. A peripheral cell of the endosperm of a dormant seed.

Figure 6. A typical cell of the endosperm of a dormant seed, killed with Corrosive Sublimate, Glacial Acetic Acid, Picric Acid and Alcohol.

" 7. A typical cell of the endosperm of a dormant seed, killed with Absolute Alcohol, Chloroform, and Glacial Acetic Acid

" 8. A typical cell of the endosperm of a dormant seed.

" 9. A typical cell of the endosperm of a seed germinated 48 hours, testa first removed.

" 10. A typical cell of the endosperm of a seed germinated until the testa had just broken open.

" 11. A typical cell of the endosperm of a seed germinated 93 hours, testa first removed.

" 12. A typical cell of the endosperm of a seed germinated 189 hours, testa first removed.

" 13. A typical cell of the endosperm of a seed germinated 360 hours.

" 14. A typical cell of the endosperm of a seed germinated 237 hours, testa first removed.

" 15. Another typical cell of the endosperm of a seed germinated 237 hours, testa first removed.

" 16. A typical cell of the embryo of a dormant seed.

" 17. A typical cell of the embryo of a seed germinated 48 hours, testa first removed (Same section as Figure 9).

" 18. A typical cell of the embryo of a seed germinated until the testa had just broken open (Same section as Fig.10).

" 19. A typical cell of the embryo of a seed germinated 93 hours, testa first removed (Same section as Figure 11).

" 20. A portion of a cotyledon of a seed germinated 189 hours, testa first removed (Same section as Figure 12).

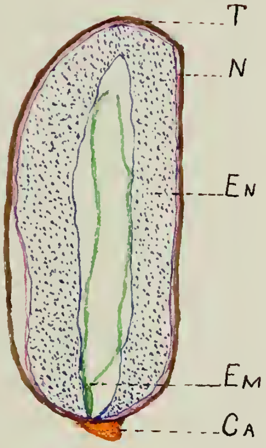


Fig. 1.

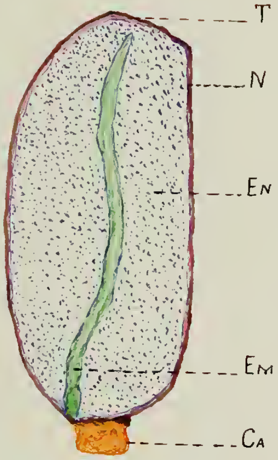
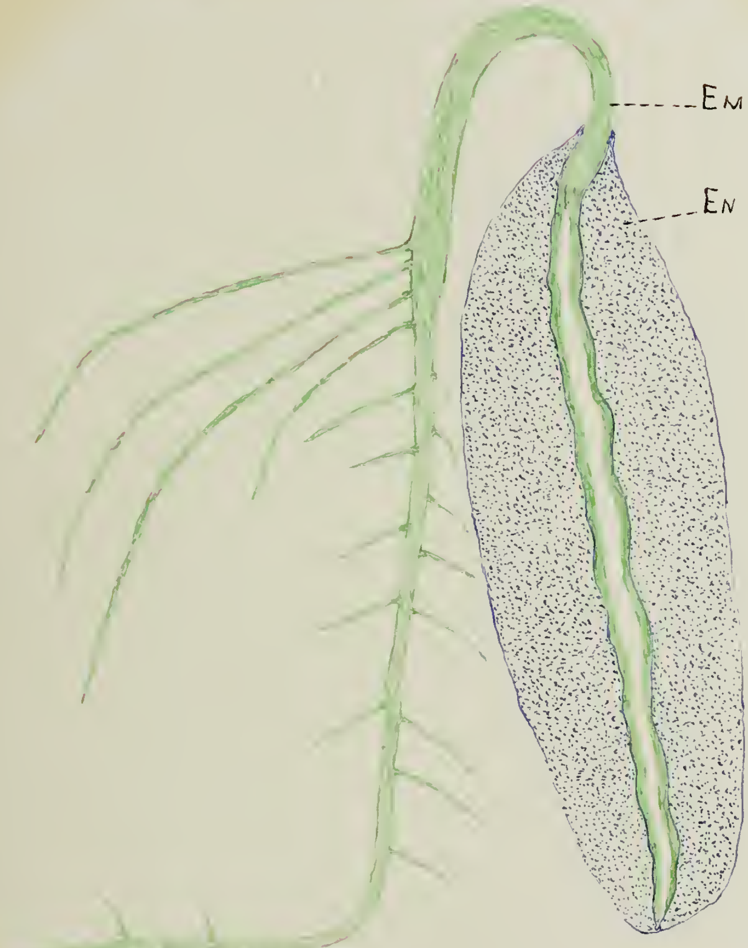


Fig. 2.



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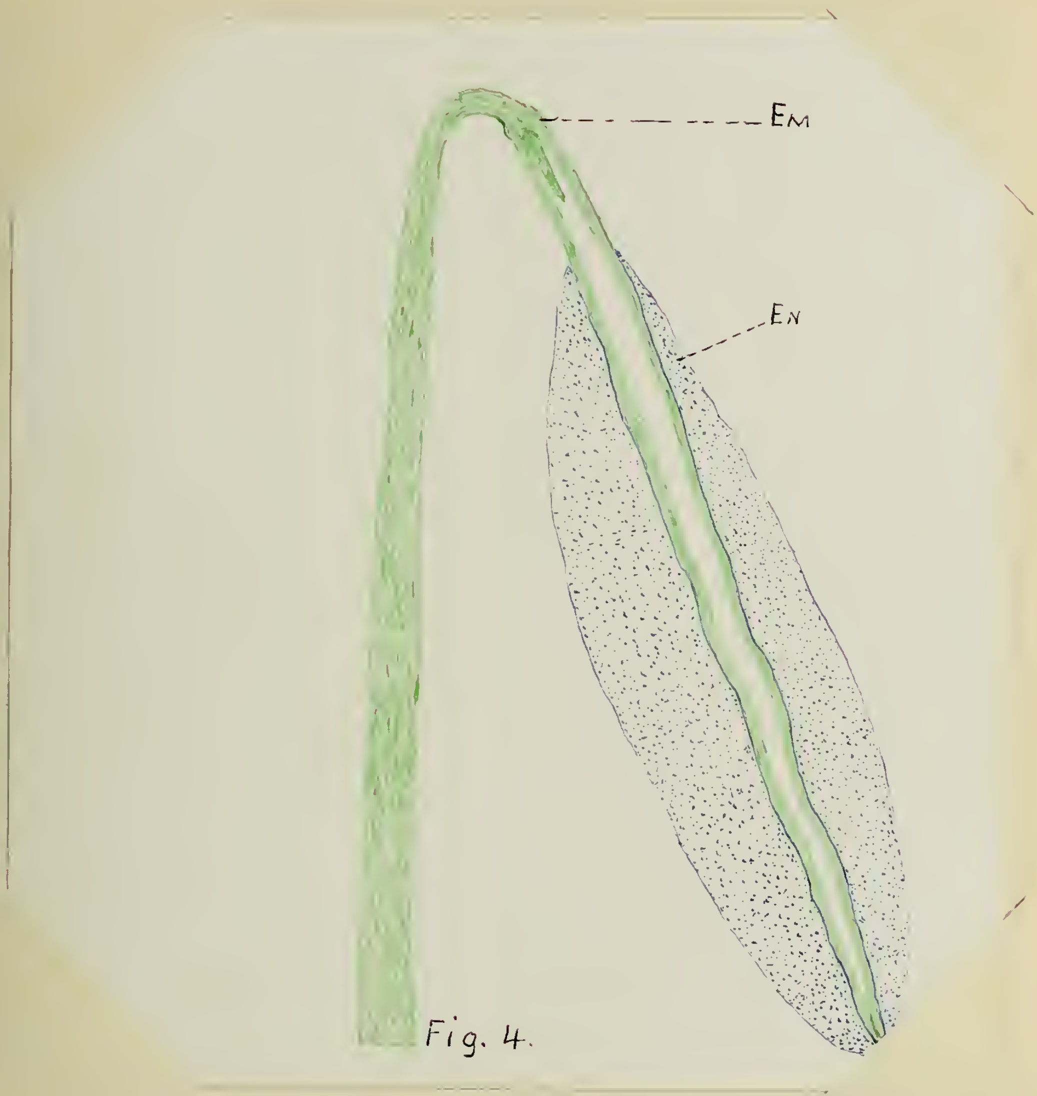

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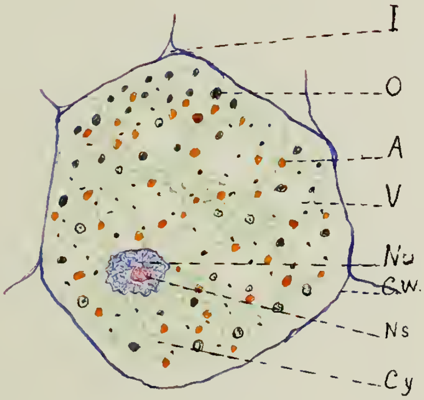


Fig. 5

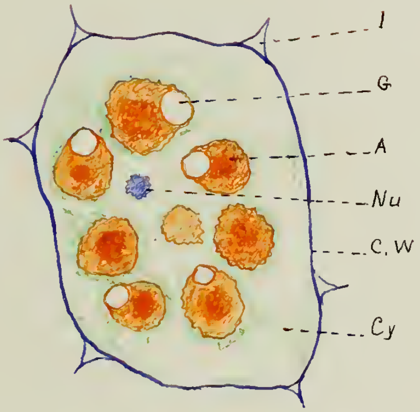


Fig. 6

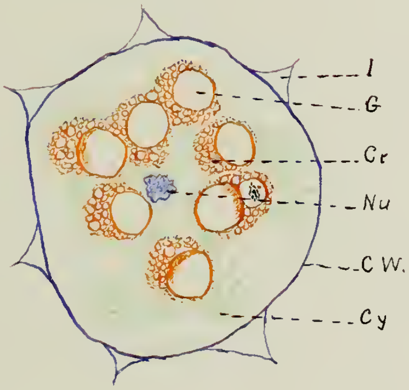


Fig. 7.

15

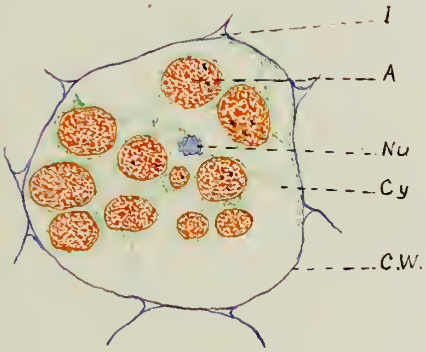
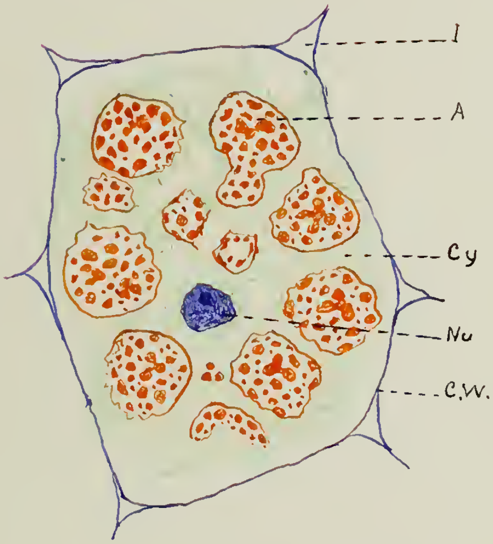


Fig. 8



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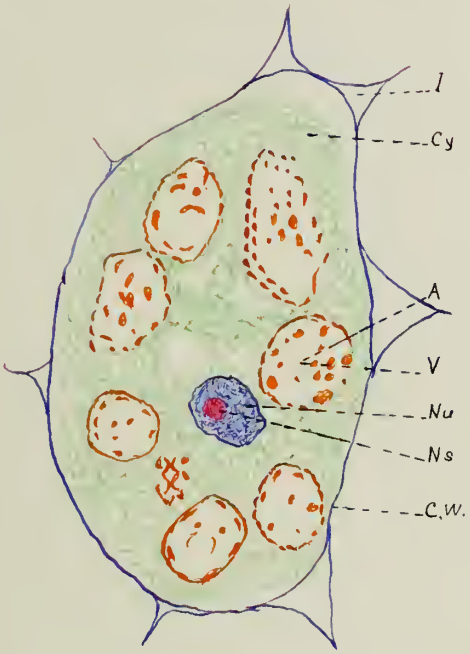
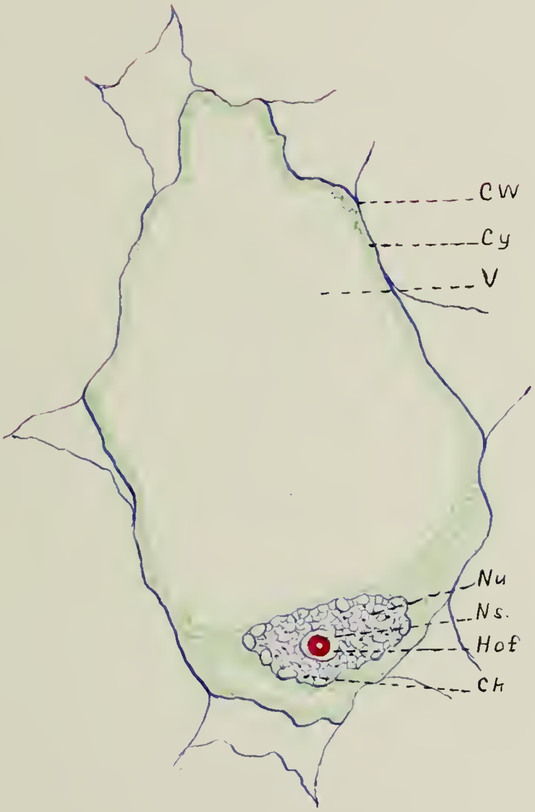


Fig. 10.

Fig. 11

Fig. 12.



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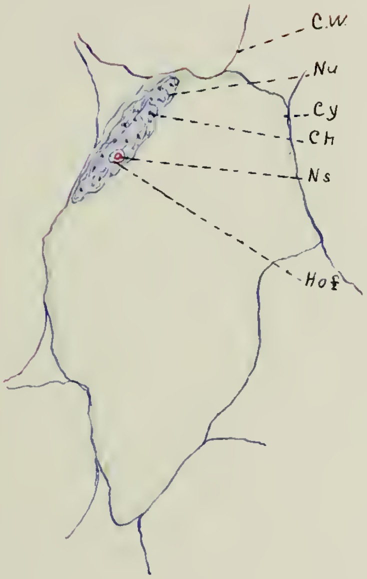

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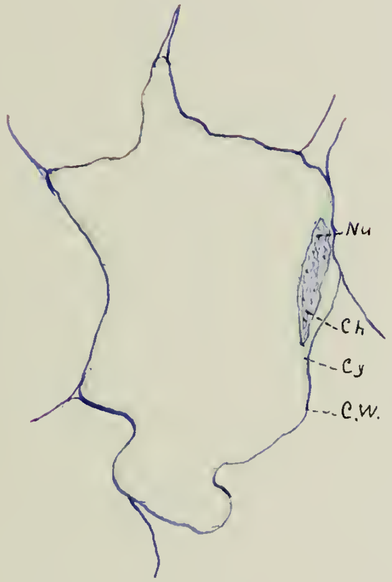
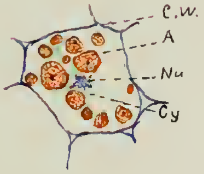


Fig. 15



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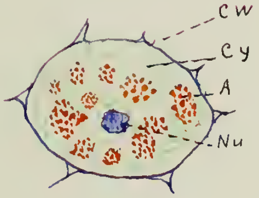

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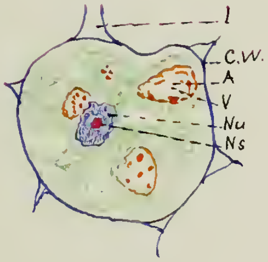

Fig. 18

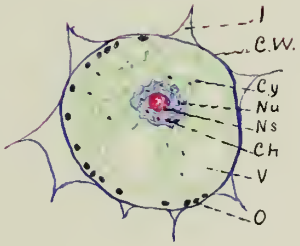

Fig. 19

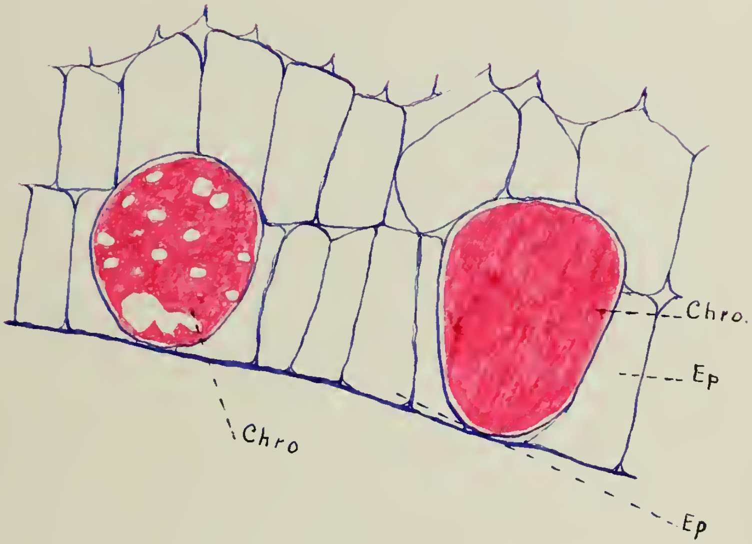

Fig. 20.